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Review

Structure, function and regulation of plant photosystem I

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Abstract

Photosystem I (PSI) is a multisubunit protein complex located in the thylakoid membranes of green plants and algae, where it initiates one of the first steps of solar energy conversion by light-driven electron transport. In this review, we discuss recent progress on several topics related to the functioning of the PSI complex, like the protein composition of the complex in the plant *Arabidopsis thaliana*, the function of these subunits and the mechanism by which nuclear-encoded subunits can be inserted into or transported through the thylakoid membrane. Furthermore, the structure of the native PSI complex in several oxygenic photosynthetic organisms and the role of the chlorophylls and carotenoids in the antenna complexes in light harvesting and photoprotection are reviewed. The special role of the 'red' chlorophylls (chlorophyll molecules that absorb at longer wavelength than the primary electron donor P700) is assessed. The physiology and mechanism of the association of the major light-harvesting complex of photosystem II (LHCII) with PSI during short term adaptation to changes in light quality and quantity is discussed in functional and structural terms. The mechanism of excitation energy transfer between the chlorophylls and the mechanism of primary charge separation is outlined and discussed. Finally, a number of regulatory processes like acclimatory responses and retrograde signalling is reviewed with respect to function of the thylakoid membrane. We finish this review by shortly discussing the perspectives for future research on PSI.
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Keywords: Excitation energy transfer; Light harvesting; Photosynthesis; Red chlorophylls; Regulation; State transitions

1. Introduction

Photosystem I is one of the two membrane-bound photosystems of plants, algae and cyanobacteria that mediate light-driven electron transport from water to NADPH. The concept of two different plant photosystems emerged in the 1960s and was mostly based on spectroscopy measurements. The first higher plant photosystem I was isolated and characterized with respect to chlorophyll content and photochemical activities as early as 1966 [1]. However, it took until 1975 before the first report of a purified plant photosystem I complex and its subunit composition was published [2]. Since then our knowledge of photosystem I has been steadfastly increased to a level where we now can ask the real questions how plants utilize light energy for their growth and maintenance under changing conditions and in particular about the role of photosystem I.

Photosystem I generates the most negative redox potential in nature and is extremely efficient in its utilization of light for electron transport from plastocyanin on the lumenal side to ferredoxin on the stromal side of the thylakoid membrane. In plants, the PSI complex consists of at least 19 protein subunits, approximately 175 chlorophyll molecules, 2 phylloquinones and 3 Fe_4S_4 clusters [3]. This complexity naturally raises

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several questions: what is the role of the many subunits? Are all subunits important for electron transport? How are the numerous subunits assembled and organized in structural terms? How are the levels and activities of the different parts of the photosynthetic systems regulated?

With the aim to answer some of these questions a Research Training Network was formed during the European Union 5th Framework Program. The title of the network was 'Molecular dissection of photosystem I structure, function and biogenesis' and included 7 partners from Denmark, Sweden, Germany, United Kingdom, Italy and The Netherlands.

The focus of the network was higher plant photosystem I and *Arabidopsis thaliana* was the main organism used due to the availability of mutant collections for reverse genetics and amenability of genetic transformation. Some work was also performed with cyanobacteria and green algae since these organisms in some cases had obvious advantages. The network has so far produced 25 joint publications and more than 80 publications involving one network team. In the following review we will describe work performed in this network and put it into context of our general knowledge of photosystem I. For more comprehensive reviews on other aspects of photosystem I we refer to recent reviews [4,5].

2. Subunit composition and structure of photosystem I

2.1. Subunit composition

Fifteen core subunits (PsaA to PsaL, PsaN to PsaP) of PSI are known so far, and its peripheral antenna, LHCI, consists of up to six Lhca proteins (Lhca1–6). Under certain conditions a LHCII trimer composed of at least two different types of Lhcb proteins (Lhcb1–2) is also associated with PSI. The most predominant type of PSI consists of the 15 core subunits and four Lhca proteins as indicated in the schematic figure shown in Fig. 1. In Table 1, the 15 known PSI core subunits and 6 known Lhca proteins are listed along with genetic and biochemical information.

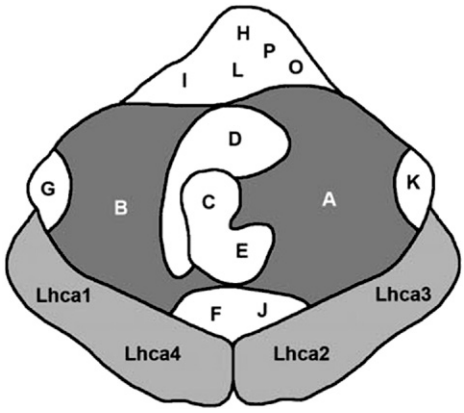

Fig. 1. Schematic figure of plant photosystem I viewed from the stromal side. The position of the PsaA–PsaL core subunits and the four Lhca subunits is based on the structural model from pea [3]. The position of PsaO and PsaP is deduced from biochemical evidence [9,50]. PsaN is located on the lumenal side and is not visible in this schematic figure.

The structural model of PSI based on X-ray crystallography diffraction reveals the precise location and structure of 12 of the 15 core subunits within the complex [3], as will be discussed in more detail in Section 2.2. For a discussion on the structural and general properties of the PSI proteins known in 2001 we refer to an earlier review [6]. Here we discuss two recently discovered PSI core proteins and two new Lhca proteins. Functional aspects of all core proteins are discussed in Section 3, those of peripheral antenna proteins in Section 5.2.

2.1.1. New core subunits of photosystem I: PsaO and PsaP

The PsaO subunit was discovered in *A. thaliana* during characterization of a mutant deficient in PsaN [7]. Like PsaN, PsaO has a size of about 10 kDa and thus the two proteins co-migrate during electrophoresis. PsaO seems to be present in higher plants, mosses and green algae but has no counterpart in cyanobacteria. The mature PsaO protein has two transmembrane helices connected by a 29 amino acid long lumenal loop and is probably located near PsaH and PsaL (see Section 3.2).

TMP14 was identified as a 14 kDa thylakoid membrane phosphoprotein with unknown function [8]. More recently it was shown that TMP14 is a PSI subunit, designated PsaP. Using blue native/SDS-PAGE it was shown that PsaP is present in the stroma lamellae where it co-migrated exclusively with the PSI complex [9]. In grana the PsaP protein was not detected. In sucrose gradient fractionation an enrichment of the PsaP protein in the PSI containing fractions was clearly seen and PsaP is also absent in the PSI-deficient barley mutant *viridis-zb*^{63} [9]. PsaP might be located close to PsaL, PsaH and PsaO (Section 3.2). Homologues of PsaP exist in other higher plants and cyanobacteria but apparently not in green algae. The similarity between plant PsaP and its cyanobacterial homologues is quite low and the cyanobacterial PSI structure contains no PsaP homolog [10] suggesting that the PsaP-like proteins found in cyanobacteria are not part of PSI, but confers a different function.

2.1.2. Novel LHCI proteins — Lhca5 and Lhca6

Four membrane-bound subunits Lhca1–Lcha4 bind cooperatively to the PSI core complex and form LHCI, the peripheral antenna of PSI. However, some heterogeneity of LHCI has been observed. In tomato, for example, isoforms of Lhca1 and Lhca4 exist. Some species, such as *A. thaliana*, have only one gene for each Lhca1–4 subunit, but have in addition two homologous genes encoding a fifth and sixth Lhca protein (Lhca5 and Lhca6) [11]. Lhca5 and Lhca6 are highly homologous to the Lhca1–4 proteins; Lhca6 was even so similar that it first was assumed to be Lhca2 when discovered. The Lhca5 protein has recently been characterized in more detail, and it seems as if Lhca5 accumulates to sub-stoichiometric amounts with respect to PSI. However, it is more abundant under certain conditions like high light and its content is rather stable in contrast to other PSI and LHCI subunits when Lhca1 and Lhca4 are depleted due to mutations [12]. Lhca5 does not have the "very red" chlorophylls that Lhca3 and Lhca4 possess [13], but it can form homodimers and seems to associate to native PSI at Lhca2 and Lhca3 and to the PSI core at the Lhca1/4 binding site [14].

Table 1
Photosystem I subunits of *A. thaliana*

Gene	Gene location [a]	Accession number [b]	Protein	Molecular mass (kDa) [c]	Cofactors [d]	Function	Mutation [e]	Antibody [f]
PsaA	C	AtCG00350	PsaA	83.2	≈79 Chl *a*, β-carotene,	Light-harvesting		AS06 172
PsaB	C	AtCG00340	PsaB	82.5	P700, A_0, A_1, F_X	Charge separation Electron transport		AS06 166
PsaC	C	AtCG01060	PsaC	8.9	F_A, F_B	Electron transport		AS04 042
PsaD	N (2)	At4g02770 At1g03130	PsaD	17.9/17.7		Binding of ferredoxin Binding of PsaC	SUP and KO	AS04 046
PsaE	N (2)	At4g28750 At2g20260	PsaE	10.4/10.5		Binding of ferredoxin and FNR Involved in cyclic electron transport	KO	AS04 047
PsaF	N	At1g31330	PsaF	17.3		Binding of plastocyanin Binding of Lhca1/4	SUP and KO	AS06 104
PsaG	N	At1g55670	PsaG	11.0	1 Chl *a* 1–2 β-carotene	Binding of Lhca1/4 Regulation of PSI	SUP and KO	AS04 048
PsaH	N (2)	At3g16140 At1g52230	PsaH	10.4/10.4	1 Chl *a*	Binding of LHCII (state transitions) Stabilization of PsaD	SUP and KO	AS06 143
PsaI	C	AtCG00510	PsaI	4.1		Stabilization of PsaL		
PsaJ	C	AtCG00630	PsaJ	5.0	2 Chl *a*	Stabilization of PsaF		
PsaK	N	At1g30380	PsaK	8.5	2 Chl *a*	Binding of Lhca2/3	SUP and KO	AS04 049
PsaL	N	At4g12800	PsaL	18.0	3 Chl *a*	Stabilization of PsaH and PsaO	SUP	AS06 108
PsaN	N	At5g64040	PsaN	9.7		Docking of plastocyanin	SUP and KO	AS06 109
PsaO	N	At1g08380	PsaO	10.1	Chl *a* ?	Binding of LHCII (state transitions)	SUP and KO	AS04 050
PsaP	N		PsaP					
Lhca1	N	At3g54890	Lhca1	21.5	13 Chl 3 carotenoids	Light-harvesting	KO	AS01 005
Lhca2	N	At3g61470 (At5g28450)	Lhca2	23.2	13 Chl 2 carotenoids	Light-harvesting	SUP	AS01 006
Lhca3	N	At1g61520	Lhca3	24.9	13 Chl 3 carotenoids	Light-harvesting	SUP	AS01 007
Lhca4	N	At3g47470	Lhca4	22.3	13 Chl 2 carotenoids	Light-harvesting	KO/SUP	AS01 008
Lhca5	N	At1g45474	Lhca5	24.3	13 Chl 2 carotenoids	Light-harvesting	KO	AS05 082
Lhca6	N	(At1g19150)	Lhca6	24.6				

Location of the gene in the chloroplasts (C) or the nucleus (N) is indicated together with the number of genes encoding the subunit in *Arabidopsis* and the accession number. Indicated are also cofactors attached to the individual subunits and function of the individual subunit in PSI. The existence of plant lines with inactivated genes and the nature of the inactivation are listed. Finally, the existence of commercially available antibodies is also indicated.

[a] The location of the gene in the nuclear (N) or plastid (C) genome in *Arabidopsis* is indicated. Number in brackets indicates the cases where there is more than one nuclear gene in the *Arabidopsis* genome encoding the subunit.

[b] Genes in parentheses have little or no expression.

[c] The predicted molecular mass of the mature *Arabidopsis* protein is indicated. The predicted masses are for the apoproteins without cofactors. Cleavage sites for signal and transit peptides were predicted by alignment with homologous sequences with known cleavage sites from other species.

[d] The numbers of Chl bound are from the structure of PSI from pea [3].

[e] The expression of the gene is either affected indirectly via sense cosuppression, antisense suppression, RNAi suppression as indicated by 'SUP' or directly by transposon or T-DNA knockout as indicated by 'KO'.

[f] The availability of antibodies from Agrisera AB, Sweden (www.agrisera.se) indicated with product number.

Data for Lhca6 are scarce, but the *Lhca6* mRNA profiles match that of Lhca5 and several of the one- and two-helix proteins homologous to the Lhc proteins, with yet unknown functions [15].

2.2. Structure of plant photosystem I

The first reliable 2D maps on plant PSI structure were only published recently. From a single particle electron microscopy study it was concluded that LHCI only binds to the core complex at the side of the PsaF/PsaJ subunits. The number of Lhca subunits comprising LHCI was estimated to be at least 4–5 [16]. The crystallization and subsequent structure determination by X-ray diffraction was a landmark achievement in plant PSI research [3]. The monomeric plant PSI structure was solved at 4.4 Å resolution, and shows 16 protein subunits, 167 Chl molecules, 2 phylloquinones and 3 iron–sulphur clusters [3]. About eight additional densities in plant PSI are likely to be Chl molecules, giving a total of 175 Chl molecules [5]. The exact positions of 12 core subunits and 4 Lhca subunits were established (Fig. 2). Three peripheral core subunits, PsaN, PsaO and PsaP, were not detected in the crystal structure. They could

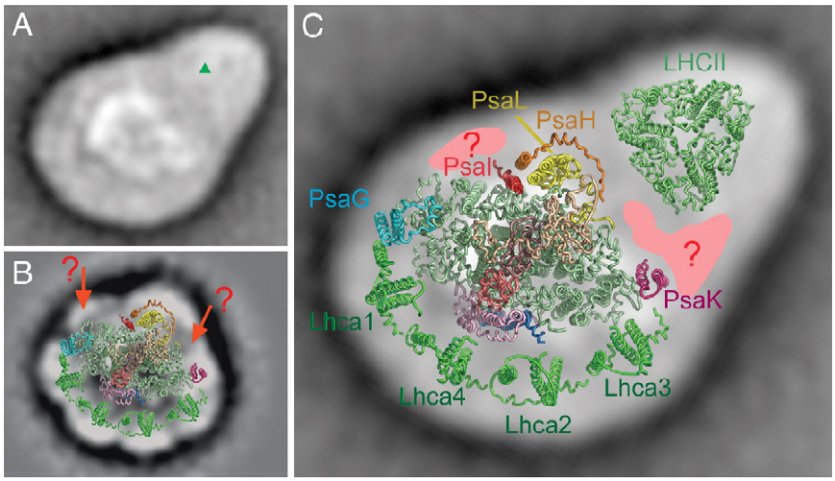

Fig. 2. Structure of plant PSI and the PSI–LHCII supercomplex viewed from the stromal side of the membrane. (A) Electron microscopy projection map of the PSI–LHCII supercomplex from *A. thaliana* at 15 Å resolution obtained by single particle averaging (improved from [120]). The position of the 3-fold axis of the LHCII trimer is indicated by a green triangle. (B) Electron microscopy projection map of the PSI complex from *A. thaliana* (S. Kereiche, R. Kouřil, E.J. Boekema, unpublished data). The atomic model pea PSI [3] has been overlayered. Red arrows indicate positions where additional density might indicate subunits lacking in the atomic model. (C) Pseudo-atomic model for the PSI–LHCII supercomplex in which the high-resolution structures of PSI and trimeric LHCII [94] have been fitted. Positions of the four peripheral antenna subunits Lhca1–4 and the small subunits PsaG, -H, -I, -K and -L closest to the LHCII trimer have been indicated. Densities in the interface of PSI and LHCII and close to PsaH and -I (pink) are not covered by the atomic structures of PSI and LHCII and likely consist of additional subunits.

have been lost during the purification and crystallization of PSI. At least the extrinsic 10 kDa PsaN subunit is easily dissected from PSI. PsaN is located on the lumenal side close to PsaF where it is involved in the docking of plastocyanin [17]. Because the PSI structure was solved at 4.4 Å resolution, which is close to the limit for assignments of polypeptide chains, the position of the four LHCI subunits Lhca1–4 was not entirely established. Additional biochemical research is in line with the originally proposed positions (Fig. 2) of the Lhca1–4 subunits [18].

The PSI–LHCI complex from the green alga *Chlamydomonas reinhardtii* is larger than that of green plants [19,20]. However, a high-resolution structure is not available yet, and considerable differences are reported for the structure of the PSI–LHCI complex of Chlamydomonas. Kargul and co-workers [21] suggested the binding of six LHCI proteins, of which four are bound at the same positions as the four LHCI proteins in green plants and two at the other side of the complex. Based on an elaborate EM analysis, we suggested the presence of nine or ten LHCI proteins [22,23], in line with biochemical data [24], of which eight or nine are bound in two rows at the PsaF/J side of the complex and one is bound at the same position at which in green plants trimeric LHCII binds in state 2 (see Section 6.2).

2.3. Photosystem I trimers?

The twelve-subunit PSI core complex structure from cyanobacteria was determined before the plant structure became available [10]. PSI occurs in trimers in cyanobacteria and prochlorophytes [25,26]. The PsaL subunit has been shown to play a role in this trimerization [27]. It was suggested that the existence of the plant-specific PsaH subunit, next to PsaL, can explain the absence of similar-shaped trimers in plants [28]. Recent blue-native gel electrophoresis studies gave evidence for the existence of dimeric and trimeric PSI complexes in green plants [29]. Single particle electron microscopy showed the presence of small numbers of trimers after solubilization with digitonin and size-exclusion chromatography [30]. The monomers integrate with their LHCI antenna, but were fuzzy, indicating a non-specific or flexible orientation. From these results it was concluded that basically all plant PSI is monomeric [30].

3. Function of the core subunits

Of the 15 core proteins only PsaA, -B, and -C are directly involved in binding the electron transport cofactors P700 (a chlorophyll dimer), A_0 (a chlorophyll *a* molecule), A_1 (a phylloquinone), F_x (a [4Fe–4S] iron–sulfur cluster), F_A and F_B (both [4Fe–4S] iron–sulfur clusters). The remainder of the protein subunits fulfill other functions: PsaF and PsaN are important for interaction with the lumenal electron donor plastocyanin [17,31,32]; PsaD and PsaE provide the docking site for soluble ferredoxin on the stromal side of the thylakoid membrane [10,33–36]. PsaF is furthermore crucial for binding of the Lhca1/Lhca4-dimer [3,17].

Biochemical and structural studies mainly in cyanobacteria have suggested that the main function of the small, membrane integral subunits such as PsaF, -I, -J, -K and -L is the stabilization of the core antenna system which consists of ~100 Chl *a* and 22 β-carotene molecules [33]. However, the small subunit composition of plant PSI is different because it is composed of at least five subunits for which equivalent subunits have so far not been found in cyanobacterial PSI: PsaG, -H, -N, -O, and -P. This suggests an additional role for the small subunits in plants. One function of plant specific core subunits is the interaction with other complexes such as LHCI and LHCII.

3.1. PsaG and PsaK

PsaG and PsaK are two small membrane intrinsic proteins of approximately 10–11 kDa each with two transmembrane α-helices connected by a stromal-exposed loop [37,38]. PsaG is unique to higher plants and algae whereas PsaK also is present in cyanobacteria. PsaG and PsaK found in plants or algae are somewhat similar. A comparison of PsaG and PsaK from *Arabidopsis* displays approximately 30% amino acid identity. Within the PSI complex PsaK is bound to PsaA and PsaG is bound to PsaB at a roughly symmetry-related position [3]. Elimination of PsaK in plants using either antisense or gene knock-out technology has demonstrated that PsaK is involved in binding of Lhca2 and Lhca3 [39–41]. In contrast to this, plants devoid of PsaG have a 20–40% reduction of PSI content but an unaffected functional antenna size of the remaining PSI [40,41], suggesting that PsaG affects stability of the PSI complex but is not strictly needed for binding of Lhca1 and Lhca4. Surprisingly, the light-dependent reduction of $NADP^+$ was 45–50% higher in the thylakoids devoid of PsaG compared to wild-type [40]. This could in part be explained by a difference in the affinity for plastocyanin since the dissociation constant (K_D) for plastocyanin is only 12 μM in the absence of PsaG compared to 32 μM for the wild-type [42]. PsaG is not located close to the Pc docking site [3] but on the other hand the 4.4 Å structural model does not reveal the docking site of Pc nor does it reveal the location of PsaN, and the interaction between PsaG and Pc could be via common interaction with another subunit, perhaps PSI-N. The effect of PsaG on Pc oxidation may also be indirect via a conformational change in PsaB that in turn affects Pc binding.

3.2. PsaH, PsaL, PsaO and PsaP

One of the surprises of the functional PSI research was the discovery that PsaH was involved in balancing of the excitation energy between PSI and PSII via state 1–state 2 transition [43]. In this process a mobile pool of LHCII moves from PSII to PSI under light conditions that favors PSII and vice versa [43–45] (see Section 6).

A structural and functional relationship between PsaH and PsaL was clearly demonstrated using cross-linking [47] and gene suppression techniques [48,49]. The O-subunit of PSI [7] was subsequently shown to bind to the PsaL/PsaH-side of the PSI-complex due to the fact that plants down-regulated in the PsaL protein retain only about 10–20% PsaH and PsaO, and primary down regulation of PsaH results in 50% reduction of PsaL and 80–90% reduction of PsaO [50]. This indicates that binding of PsaO to the PSI complex is dependent on the presence of both PsaH and -L. Interaction between PsaO and PsaL was furthermore confirmed by chemical cross-linking. A peculiar observation was that the amount of the PsaL subunit was increased in plants devoid of PsaO. These observations fit very well with PsaL being the most ancient and conserved subunit closest to the core of PSI and the eukaryotic PsaH and PsaO proteins being later additions located more peripherally in the complex. In accordance with this, plants lacking PsaO have a 50% reduction in state transitions indicating a role for PsaO in the balancing of excitation energy between the two photosystems. The amount of the recently discovered PsaP subunit [9] was demonstrated to correlate with the amount of PsaL, i.e. absent in plants lacking PsaL, reduced in plants devoid of PsaH and increased in plants lacking PsaO.

Based on these observations a functional model of PSI is proposed in which PsaL as the most ancient subunit is closest to the PsaA/B-core of PSI and PsaO is positioned next to PsaL on the PsaH/L/I side of the PSI complex (Fig. 1). PsaP might also be associated with the PsaL side. Cross-linking revealed that the docking site of LHCII on PSI is comprised of at least the PsaH, -L and -I subunits [51]. Functional antenna size measurements further showed that plants devoid of PsaH or PsaL have smaller antenna in state 2 when compared to wild-type. Thus, PsaH, -L, -O, -P and possibly -I are all involved in forming a domain in PSI which is involved in interaction with LHCII. Some of the proteins interact directly with LHCII and others may be required for the stability or assembly of the domain (see also Section 6.2).

3.3. PsaJ

PsaJ is a hydrophobic subunit of 6 kDa with one transmembrane helix that is located close to PsaF [3,10]. The N-terminus of PsaJ is located in the stroma; the C-terminus is located in the lumen [10]. In cyanobacteria, PsaJ binds three chlorophylls [10] whereas in plants, PsaJ only binds two chlorophyll molecules [3].

In studies using *Chlamydomonas* without PsaJ it was concluded that in 70% of the PSI complexes lacking PsaJ, the N-terminal domain of PsaF is unable to provide a binding site for either Pc or Cyt c_6 that in turn lead to electron transfer to $P700^+$ and was explained by a displacement of this domain [52]. Thus, PsaJ does not appear to participate directly in binding of Pc or Cyt c_6 but plays a role in maintaining a precise recognition site of the N-terminal domain of PsaF required for fast electron transfer from Pc and Cyt c_6 to PSI.

Tobacco plants with an inactivated *psaJ* gene are slightly smaller and paler than wild-type due to an approximate 20% reduction in photosystem I [53]. The specific PSI activity measured as $NADP^+$ photoreduction in vitro revealed a 55% reduction in electron transport through PSI in the absence of PsaJ. Immunoblotting analysis revealed a secondary loss of the lumenal PSI-N subunit in PSI particles devoid of PSI-J. Presumably PSI-J affects the conformation of PSI-F which in turn affects the binding of PSI-N. This together renders a fraction of the PSI particles inactive. Thus, PSI-J is an important subunit that together with PSI-F and PSI-N is required for formation of the plastocyanin binding domain of PSI. PSI-J is furthermore important for stability or assembly of the PSI complex.

Inactivation of genes encoding subunits in PSI either by suppression technology or gene inactivation has proven a very useful approach to study their function within the complex. The attenuation of gene expression using suppression technology or elimination, of one of the genes in cases where the subunit are encoded by two genes, has even allowed functional

analysis of vital subunits such as the PsaD, -E and -F subunits [17,34–36].

4. Targeting of PSI proteins to the thylakoid membrane

The biogenesis of the PSI complex is a complex issue. The complex includes a large number of integral membrane proteins, together with hydrophilic subunits on both the stromal and lumenal sides of the membrane. Most of these proteins are synthesised in the cytosol and they must therefore be transported across the envelope membranes, through the stromal phase and into or across the thylakoid membrane to the correct location. In addition, some PSI subunits (PsaA, -B, -C, -I and -J) are encoded by chloroplast genes, further complicating the overall assembly process. Clearly, the assembly of this complex requires the operation of highly efficient mechanisms for the targeting of the constituents to the correct location within the chloroplast.

Relatively little is known about the insertion of chloroplast-encoded thylakoid membrane proteins and the biogenesis of this set of PSI component (including the core PsaA and PsaB subunits) is thus poorly understood. However, much more is known about the biogenesis of the nuclear-encoded PSI subunits and this section will focus on the targeting pathways involved, with a particular emphasis on the events occurring at the thylakoid membrane. Of course, the biogenesis of the PSI complex additionally requires an efficient system for assembling the numerous subunits into the holocomplex, but these processes will not be covered here.

4.1. Translocation across the chloroplast envelope

The import of chloroplast proteins occurs post-translationally and the vast majority of stromal and thylakoid proteins are imported by a common default pathway. In this pathway, the imported protein is synthesised with a cleavable N-terminal presequence, often termed the transit peptide, and import is mediated by the concerted action of protein translocation systems in the outer and inner envelope membranes. These translocases are referred to as the Toc and Tic systems (for *T*ranslocase of the *O*uter/*I*nner *C*hloroplast membrane). The import pathway has been extensively reviewed [54]. Briefly, the precursor protein docks onto specific receptors on the outer membrane (Toc159 and Toc34). These subunits are GTPases and there is evidence that GTP hydrolysis leads to partial insertion of the proteins into the protein-conducting channel (Toc75). Further translocation involves interaction with the Tic apparatus and the protein is threaded through the membrane in an unfolded state with the aid of ATP hydrolysis in the stroma.

After import into the stroma, the PSI proteins undergo further targeting to the thylakoid membrane. Surprisingly, at least four mainstream pathways have been identified for the targeting of proteins into and across the thylakoid membrane, and all four are used by PSI proteins. This complexity of intraorganellar protein sorting is in stark contrast to the operation of a single primary pathway for the import of so many proteins (around 3000) from the cytosol.

4.2. Insertion of thylakoid membrane proteins

The insertion of hydrophobic membrane-spanning proteins has been intensively studied in bacteria, especially *Escherichia coli*, and it is known that the vast majority of plasma membrane proteins are inserted by the signal recognition particle (SRP) pathway. In this pathway, the nascent membrane protein is recognised by SRP, which is a complex of a 54 kDa protein (SRP54) and small RNA molecule. The SRP next interacts with a partner protein, FtsY, and the two factors deliver the substrate to a membrane-bound protein translocase (SecYEG complex). A key role is played by an additional factor, YidC, which appears to work alongside the SecYEG complex and markedly increase the efficiency of the insertion process. Insertion takes place co-translationally (reviewed by [55]).

The discovery of an SRP-dependent pathway in chloroplasts [56] was unsurprising because these organelles are generally believed to have evolved from endoosymbiotic cyanobacteria. Initially, it was expected that this would be a default mechanism for the insertion of thylakoid membrane proteins, but subsequent studies have generated some major surprises.

First, the chloroplast SRP has a unique structure. Initial studies on this pathway involved the use of *in vitro* assays for the insertion of the major PSII light-harvesting chlorophyll-binding protein (Lhcb1) into isolated thylakoids. It was found that insertion required a stromal SRP containing a homologue of bacterial SRP54 subunits, but this SRP is otherwise very different in that (i) it does not contain RNA and (ii) it contains a novel 43 kDa subunit (SRP43) which appears to be unique to chloroplast SRPs [57]. Other aspects of the pathway appear to be broadly similar to those of bacterial SRP pathways: the involvement of FtsY has been demonstrated [58] and a YidC homologue, Alb3, is required for the insertion of the SRP substrate into the thylakoid membrane [59]. However, a major difference may be a lack of involvement of the thylakoid SecYE complex, although this important point remains to be confirmed.

Subsequent studies have shown that the SRP pathway is used for other components of the LHCII complex, and there is some evidence that the pathway is also used for the related subunits of the LHCI complex; a *C. reinhardtii* mutant lacking Alb3 is almost devoid of LHCI [60]. The initial expectation was that essentially all thylakoid membrane proteins would turn out to be targeted by the SRP pathway, but the second main surprise in this field has been the realisation that, in terms of number of substrates, it is in fact a specialised minority pathway.

4.3. A highly unusual, possibly spontaneous mechanism for thylakoid membrane protein insertion

Many thylakoid membrane proteins have been studied using *in vitro* insertion assays, and the vast majority uses an insertion mechanism that is very different to the SRP-dependent mechanism used by light-harvesting chlorophyll-binding proteins. Critically, insertion does not rely on nucleoside triphosphate hydrolysis, SRP or any other stromal factor. This pathway for membrane protein insertion is highly unusual, if not unique, since almost every other protein transport/insertion

pathway requires energy in the form of nucleoside triphosphate hydrolysis or a proton motive force. It has also been shown that the YidC homologue, Alb3, is not required for insertion, and no other candidate proteinaceous receptor has been identified, strengthening the possibility of a purely spontaneous insertion pathway. Focusing on PSI subunits, PsaK and PsaG have been shown to insert via this mechanism, and there appears to be little doubt that other nuclear-encoded subunits follow this pathway [37,61,62].

4.4. Two pathways for the targeting of nuclear-encoded lumenal PSI subunits

Two very different pathways are also used to transport soluble proteins across the thylakoid membrane (reviewed by [54]). One is related to Sec-dependent export systems in bacteria; here, the lumenal protein is synthesised with a bipartite presequence containing a transit peptide followed by a classical 'signal peptide'. After removal of the transit peptide in the stroma, the signal peptide directs transport by the Sec machinery. The substrate interacts with SecA in the stroma, which hydrolyses ATP and drives translocation through a membrane-bound SecYE complex [63]. PsaF has been shown to use this pathway; strictly speaking this is not a lumenal protein since it contains a C-terminal transmembrane span, but the remainder of the protein is in the form of a large globular domain in the lumen.

Other lumenal proteins are transported by the twin-arginine translocation (Tat) pathway. The Tat systems differs in fundamental respects from the Sec pathway and studies on both the thylakoid and bacterial systems have provided compelling evidence that it is able to transport fully folded proteins by a wholly novel mechanism [reviewed by 54]. The system appears not to rely on stromal factors or nucleoside triphosphates, and *in vitro* studies on chloroplasts and isolated thylakoids have shown an absolute requirement for the thylakoidal ΔpH [64]. In bacteria, one of the known roles for the Tat system is to transport proteins containing any of a range of redox cofactors, such as FeS or molybdopterin centres. However, other substrates do not bear cofactors and it appears that the Tat system is also used for substrates that simply fold too tightly or rapidly for the Sec pathway to handle. Among PSI subunits, the lumenal PsaN subunit has been to shown to follow the Tat pathway [65].

In summary, PSI proteins appear to be imported into the chloroplast by a single pathway but they are subsequently transported into the thylakoid by a multitude of pathways. True membrane proteins are targeted by the SRP and 'spontaneous' pathways, with the Lhca subunits believed to follow the former and other subunits, including PsaK, PsaG and probably PsaO, following the latter. The lumenal (or predominantly lumenal) proteins are transported by the ATP-dependent Sec pathway (in an unfolded state) or the ΔpH-dependent Tat pathway, which appears to transport folded proteins. Further work is required to understand the targeting of the chloroplast-encoded PSI subunits, which may well follow additional pathways or variants of the above.

5. Function of the Photosystem I antenna

5.1. Core antenna

The antenna of PSI consists of two structurally and functionally parts: the core antenna and the peripheral antenna. The core antenna consists of about 100 Chl *a* and 20 β-carotene molecules [3,10]. The chlorophylls have their Q_y absorption maxima around 680 nm. Most of the chlorophylls and carotenes are bound to the main subunits PsaA and PsaB, but also some of the small subunits bind a few pigments. A comparison of absorption spectra of PSI–LHCI complexes from wild type *A. thaliana* and from a mutant lacking the PsaL and PsaH subunits revealed that the about five chlorophylls that are bound to these subunits absorb preferentially at 688 and 667 nm [66]. The PSI core antenna also binds so-called red chlorophylls (chlorophylls that have their absorption maxima at longer wavelengths than the primary electron donor P700), and it is now clear that the numbers and absorption characteristics of red chlorophylls vary strongly in PSI core complexes from different organisms [67,68]. In green plants, red chlorophylls also occur in the peripheral antenna, and because these chlorophylls are more abundant in the peripheral antenna and have lower energies, we will in this paper focus the discussions on red chlorophylls on those in the peripheral antenna—see Section 5.2.3. For general discussion of the spectroscopic properties of the core antenna chlorophylls we refer to recent reviews [69,70].

5.2. Peripheral antenna

First evidence for the existence of a Chl *a/b* antenna specifically associated to higher plants PSI was provided by the work of Mullet et al. [71] which identified a fraction containing four polypeptides with molecular mass between 20 and 24 kDa as belonging to light harvesting complex of Photosystem I (LHCI). LHCI was later isolated in two different pigment–protein fractions: one, monomeric, was enriched in Lhca2 and Lhca3 while another, dimeric, contained Lhca1 and Lhca4 [72]. According to their emission peaks at low temperature, the two fractions were named LHCI-680 and LHCI-730 respectively [73–76]. In some cases, however, it was possible to purify LHCI fractions containing all four Lhca polypeptides [77,78]. The recent resolution of the PSI–LHCI structure [3] provided explanation for these different experimental evidences: gap pigments at protein–protein interfaces stabilize the interactions between LHCI subunits and between these and the PSI core. Thus, relatively harsh detergent treatments are required for antenna dissociation that easily leads to partial denaturation of the pigment–protein complexes.

LHCI isolated by milder procedures is dimeric and has emission forms at 702 and 730 nm [79,80]; the absence of 680 nm emission indicates that such emission is not present in the native PSI antenna and that LHCI-680 was a partially denatured state of an originally dimeric complex with a longer fluorescence emission. Therefore, the names LHCI-680 and LHCI-730 are misleading and should not be used. Rather, the two heterodimers both containing chlorophylls

emitting around 730 nm could better be named Lhca1/4 and Lhca2/3.

5.2.1. Pigment binding of LHCI

Since native LHCI complexes are so hard to purify, an alternative approach has been used to study LHCI: *in vitro* reconstitution of recombinant proteins. This method exploits the ability of Lhc apoproteins to refold *in vitro* in the presence of chlorophylls and carotenoids [81] and allowed biochemical and spectroscopic characterization of the individual Lhca proteins [79,82–84].

This work has showed that Lhca proteins can be grouped into two pairs with respect to their pigment binding characteristics. The first group consist of Lhca1 and Lhca3 which have high affinity for Chl *a*, as reflected by their Chl *a/b* ratios of 4.0 and 5.9 in *Arabidopsis* [79,84] and consistently, Chl *b* is not essential for their refolding *in vitro* [83]. Both Lhca1 and Lhca3 bind 3 carotenoid molecules per polypeptide, mainly lutein and violaxanthin. The other group, Lhca2 and Lhca4, have lower Chl *a/b* ratios (1.9 and 2.4, [84]), cannot fold without both Chl *a* and Chl *b* [83] and bind only two carotenoid molecules per polypeptide, again lutein and violaxanthin. This grouping is also reflected in sequence homology: Lhca2 and 4 are more similar to each other than to Lhca1 or 3 [11].

Only reconstitution *in vitro* of the Lhca1/4 dimer has been achieved, the Lhca2/3 heterodimer was never obtained [82,83], preventing direct comparison between different dimers. However, a partial purification of Lhca1/4 from Lhca2/3 can be obtained by isoelectrofocusing [79] or purification of LHCI from mutants depleted in individual Lhca proteins [85]. Obviously, properties of the Lhca2/3 dimer are very similar to those of Lhca1/4 dimers, since no major differences in biochemical or spectroscopical properties of these fractions are observed. Thus, although the properties of the individual polypeptides are different, the two heterodimers which actually form LHCI are, instead, very similar.

Only 10–11 tightly bound chlorophylls are present in complexes refolded *in vitro* [79] while additional 1–2 chromophores are present *in vivo* [3]. Moreover, the binding of linker chlorophylls bridging Lhca subunits are not expected to be preserved in monomeric Lhca complexes. Data from recombinant proteins, thus, need to be integrated by *in vivo* analyses to obtain information on the additional pigment binding sites, as demonstrated by Klimmek et al. [86].

5.2.2. A spectroscopic peculiarity of LHCI: The "Red Chlorophylls"

The long wavelength emission mentioned above has a considerable effect on the fluorescence emission spectrum of thylakoids: at 77K the two main emission peaks are at 685 nm and at 735 nm. The peculiar PSI–LHCI emission originates from Chl *a* molecules absorbing at wavelengths over 700 nm, implying these chlorophylls undergo the strongest absorption spectral shift so far observed. For this reason they are often named "red chlorophylls" or "red spectral forms". The "red forms" are found in the PSI core in all organisms studied so far [69], but in the case of vascular plants the red-most Chls, emitting at 735 nm, are located in the antenna moiety rather than in the core complex whose red-most emission is at 720 nm [71,77].

Mutant plants depleted in individual Lhca polypeptides and reconstitution *in vitro* has been used to determine the location of the red Chls. Lhca4 was the first Lhca gene product shown to contain "red forms" [82,87,88]. Secondly, red-shifted emission was found to be associated with Lhca3 as well, although its fluorescence emission, at 725 nm, is slightly less red-shifted than that in Lhca4 [83,84,89]. Now, red -shifted Chls have been identified also in Lhca1 and Lhca2, emitting at 701–2 nm (Fig. 3A and B).

Red chlorophylls are also present in monomeric pigment-proteins reconstituted *in vitro* but the total amplitude of "red absorption" in all monomeric Lhca is lower than in the intact PSI–LHCI supercomplex. Consistently, in both isolated and reconstituted Lhca proteins, emission forms from both bulk and red-shifted Chls are readily detectable, while in PSI–LHCI spectra only red-most forms can be observed. A reduction in total "red absorption" is also evident when comparing dimeric Lhca1/4 to its monomeric moieties [79] or PSI–LHCI supercomplex to isolated LHCI and PSI core [85]. It seems as if multiple interactions established by Lhca subunits within PSI–LHCI with neighbor protein and pigment molecules enhance the red-forms. The molecular basis of such effect may be the ability of Lhca proteins of assuming either conformation with red-shifted spectral forms or without them. The interactions with gap pigments and core subunits would stabilize the red-form-containing conformation [85].

While the fluorescence from red chlorophylls is easily detected – especially at low temperature – the elucidation of their absorption characteristics is not straightforward since they may only represent 5% of the total absorption in the Qy region [80]. Although absoption of the "red forms" are hard to detect even at 4K, site-selected fluorescence measurements on purified dimeric LHCI indicated that it peaks at 711 nm [80]. Also, analysis of site directed mutants depleted in red forms (see below) suggested that the "red" absorption is located between 700 and 705 nm for both monomeric Lhca3 and Lhca4 [90]. Similarly, the band responsible for the 701 nm emission of Lhca1 and Lhca2 complexes was located at 686–690 nm [91,92].

5.2.3. The molecular basis of the "red chlorophylls"

In vitro reconstitution is the method of choice to dissect the origin of the red chlorophylls. Conserved Chl binding residues within the polypeptide sequences of Lhca1–4 could be identified by comparison with the structurally well characterized major LHCII complex [93,94] and mutant proteins can be obtained by changing these polar residues into non polar ones, unable to coordinate the Mg^{2+} ligand of chlorophyll molecules, thus obtaining complexes depleted in specific pigments. This analysis has recently been completed for all Lhca1–4 proteins from *A. thaliana* [18,91,92,95]. Interestingly, the red chlorophylls are located at the same binding sites in all four proteins: sites A5 and B5 (603 and 609 following the new nomenclature from [94]). The presence of other pigments (Chl B6, Chl A4 and

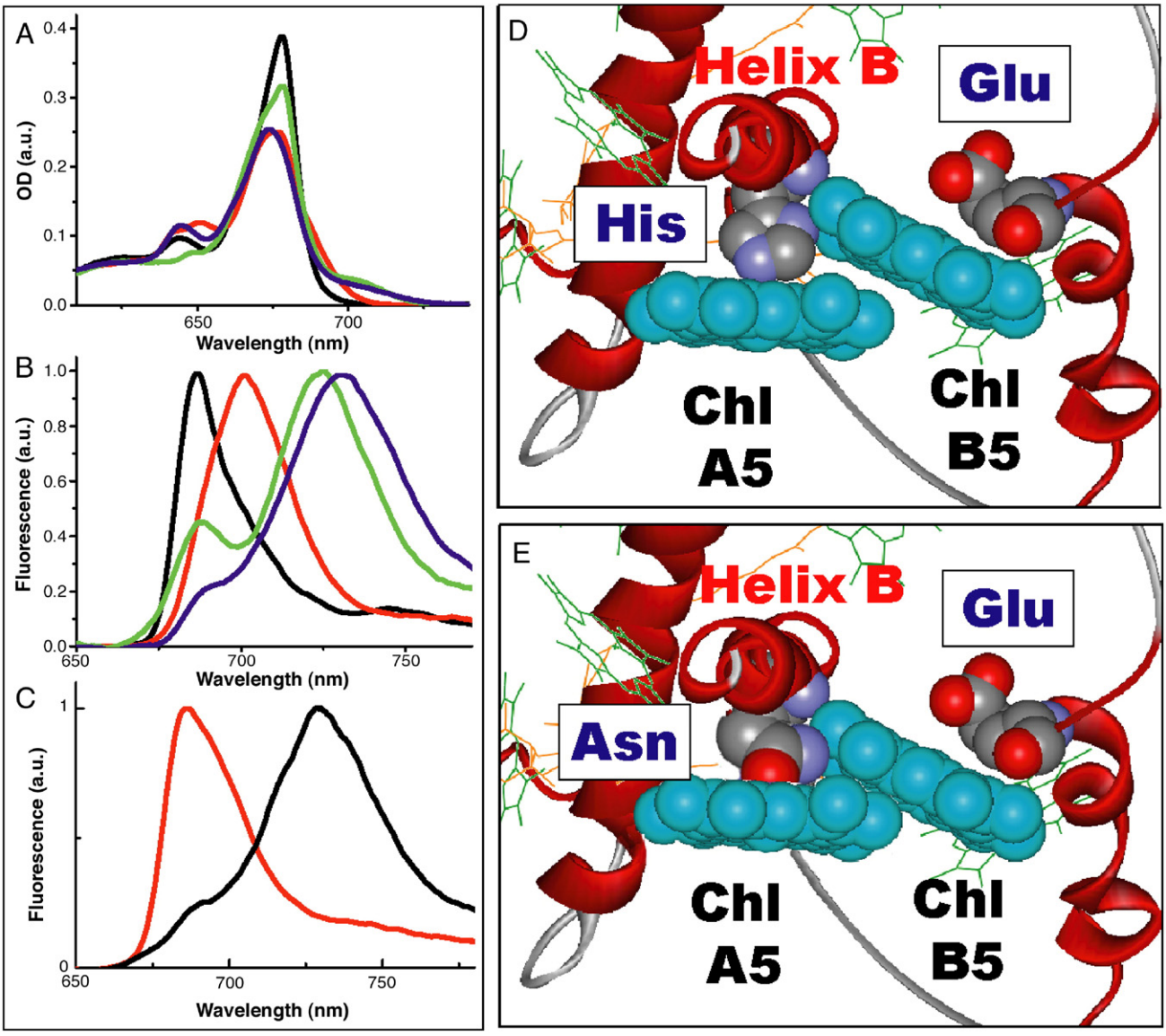

Fig. 3. Absorption spectra in the Q_y region (A) and fluorescence emission (B) at 77 K of Lhca1 (black), Lhca2 (red), Lhca3 (green), Lhca4 (blue) from *A. thaliana* reconstituted *in vitro*. Absorption spectra are normalized to the Chl content while fluorescence is normalized to the emission maxima. (C) Fluorescence emission spectra at 77K of Lhca4 WT (black) and N47H mutant (red). Spectra are normalized to the maximum. (D) Organization in the mutants, where Asn is substituted by His; (E) organization in the WTs, where the coordination of Chl A5 by Asn cause the Chl to come closer to Chl B5. Data for figure A–B and C–E are from [84,90, respectively].

the carotenoid ligand in site L2) affects either the amplitude or the energy level of red forms, although in an indirect way. The disappearance of a conservative CD signal upon mutations at sites A5 and B5 suggested that red chlorophylls originate from an excitonic interaction between two Chl *a* molecules bound to the these sites [90,91]. There might be other contributions to the large energy shift observed in the red chlorophylls although no evidences of additional components has emerged so far [18].

The presence of the red-most forms of fluorescence emission (725 to 735 in Lhca3 and Lhca4) is linked to Asn – instead of His – being ligand to Chl A5, replacing this Asn with His in Lhca3 (N62H) and Lhca4 (N47H) does not affect pigment binding but removes the red chlorophylls [90]. From the analysis of CD and LD spectra of WT and mutant, it appears as if this is due to an increased distance between Chls A5 and B5 in the mutant complexes, reducing the strength of the interaction between the two Chl molecules ([90], Fig. 3D–E). This is supported by the structure of PSI–LHCI supercomplex: Chls A5–B5 are 8 Å apart in Lhca4 (having red forms) while their distance in Lhca2 (without red forms) is 10 Å [3]. The calculated differences in interaction energy resulting from this 2 Å difference can explain the shift [92]. The reverse experiment can also be done, a Lhca1–H47N mutant proteins has a somewhat increased red emission, although the effect is incomplete due to sub-stoichiometric binding of the Chl at site A5, probably due to steric hindrance [90]. Red forms does not necessarily result from having Asn, rather than His, residues at a chlorophyll binding site, as the A2 site of the PSII Lhc proteins Lhcb1, 2, 3 and 5 has Asn residues, without possessing red chlorophylls.

Why have plants got red chlorophylls in LHCI? This we still do not know, but it has been hypothesized that they could be especially important in leaves under a canopy [96], or involved in photoprotection of PSI [97]. Since they have energy levels lower than the reaction centre most of the excitation energy populates these states even at RT [98]. As a consequence, it is possible that excess energy could be dissipated in the antenna before being transferred to the reaction center.

6. State transitions—LHCII interaction with PSI

Short-term adaptations of photosynthesis to changes in light quality and quantity include re-location of parts of the antenna complexes—leading to so-called state transitions [44–46]. State transitions balance the energy distribution between PSI and PSII, and involve the reversible association of the mobile pool of LHCII with either PSII (state 1) or PSI (state 2). When excitation of PSII is favored, and under low light conditions, the mobile LHCII pool becomes phosphorylated and attaches to PSI [99–101]. When PSI is preferentially excited, and under strong illumination, LHCII is de-phosphorylated and re-attaches to PSII. Although the mobile pool of LHCII is significantly smaller in vascular plants (15–20%) than in green algae (~80%) [101,102], state transitions are thought to function similarly in all LHCII-containing organisms [103].

6.1. State transitions require LHCII phosphorylation

LHCII can be phosphorylated by a thylakoid protein kinase. When PSII is preferentially excited, the cytochrome b_6/f complex (cyt b_6/f) interacts with the LHCII kinase and activates it [46,104–107]. A second regulatory mechanism is mediated by the ferredoxin–thioredoxin system, and down-regulates LHCII phosphorylation under high light intensities [108,109]. This involves conformational changes in the LHCII kinase induced by the reduction of thiol groups [110]. Both plastoquinone reduction and binding of plastoquinol to cyt b_6/f are required to induce LHCII phosphorylation [105,110,111], while PSII core phosphorylation requires only reduction of plastoquinone [110,112]. This supported the hypothesis that distinct kinases phosphorylate PSII core proteins and LHCII; the identity of the kinases, however, had been a matter of long-standing debate [113,114]. In 2003, the protein kinase STT7 of the green alga *C. reinhardtii* was shown to be required for LHCII phosphorylation and state transitions [115]. In *A. thaliana* two homologues of STT7 exist, STN7 and STN8 [116,117]. While the STN8 protein kinase is necessary for the phosphorylation of the PSII core proteins D1, D2, CP43 and PsbH [117,118], STN7 is required for LHCII phosphorylation and state transitions in *A. thaliana*, thus representing a functional homolog of STT7 [116]. Because only in the *stn7 stn8* double mutant the phosphorylated forms of LHCII and PSII core proteins are completely absent, STN7 and STN8 must exhibit some degree of overlap in their substrate specificities [117]. However, the clear distinction between the phosphorylation phenotypes of the two mutants implies that STN7 and STN8 act in parallel and could be directly responsible for phosphorylating LHCII and PSII core proteins [117]. Further biochemical analyses are required to unambiguously clarify whether STN7 and STN8 are sufficient for phosphorylating LHCII and PSII core, respectively, or whether phosphorylation cascades involving several kinases acting in series exist.

6.2. PSI–LHCII supercomplex

In State 2, LHCII is functionally coupled to PSI. This normally transient PSI–LHCII supercomplex appears even to be stable in plants with altered PSI complexes because of down-regulated PsaE expression [119], although the molecular basis for the accumulation of such PSI–LHCII supercomplexes under these conditions remains unclear. The docking site of LHCII on PSI is comprised of the PSI-H, -L, and -I subunits [51] but despite ample evidence for a functional interaction between LHCII and PSI, direct evidence for a physical complex and the precise docking site of LHCII on PSI has been difficult to demonstrate. It is likely that such complexes are too labile to be purified by common chromatography procedures. However, by application of single particle EM on digitonin solubilized, non-purified complexes from thylakoids prepared from plants in State 2, a 2D projection map of the PSI–LHCII supercomplex was obtained (Fig. 2A, improved from [120]), indicating the position of the trimeric position more precisely (green triangle; Fig. 2A). Modeling of the PSI [3] and the LHCII trimer structure [94] furthermore indicates how they are in direct contact with the PSI complex (Fig. 2C). The LHCII trimer is attached at a defined position close to the subunits PsaA, -H, -L and -K (Fig. 1C). This position is in line with data on *Arabidopsis* plants without the PSI-H and PSI-L subunits which are highly deficient in state transitions [43] and thus confirms the role of PSI-H and PSI-L in the binding pocket. There is, however, some space left at the interface of PSI and LHCII (pink areas, Fig. 2C) and also at the upper left periphery of the PSI core, which must contain other protein components. The presence of additional subunits at the periphery is also suggested from a 2D electron microscopy map of PSI without the additional State-2 proteins attached (red arrows, Fig. 2B). PsaO is a likely candidate because it is directly involved in state transitions [50]. The improved fitting of the PSI–LHCII supercomplex shows that PSI-K does not interact directly in the binding of LHCII, consistent with the finding that PSI-K is not necessary for state transitions [39].

PsaI has also been found to cross-link to LHCII [51]. Modeling of the PSI and LHCII trimer indicates that PsaI is not in direct contact with the PSI complex making it possible that there is a second, even weaker, binding site of LHCII that has not yet been detected. If a second binding site exists, it may be present at the symmetry-related position covered by the PSI-H, -I, -B and -G subunits. Further work is needed to clarify this point.

In the green alga *C. reinhardtii*, an analysis of detergent-solubilized PSI–LHCII particles prepared in state 1 or state 2 revealed no differences in the positions of the LHCI proteins (A. E. Yakushevska, unpublished observations). This suggests that the binding site of LHCII on green plant PSI is always

occupied by a monomeric LHC type of protein in PSI of Chlamydomonas. It was found that in state 2 the PSII protein CP29 (Lhcb4) is bound to the tip of the PSI–LHCI complex [21,121], and that of all PSII proteins, CP29 has the largest number of phosphorylation sites [122]. A binding site of CP29 on plant PSI has not been detected thus far. An analysis of antisense or knockout mutants of PSII peripheral antenna proteins has indicated that the formation of PSII–LHCII supercomplexes is prevented by the absence of CP29 [123], while the absence of other proteins of this family does not prevent the formation of PSII–LHCII supercomplexes [123,124]. So it is possible that the large extent of the state transitions in Chlamydomonas is not primarily caused by a much larger antenna size of PSI, but more by a much smaller antenna size of PSII because of the extensive phosphorylation and binding to PSI of a protein that plays a dominant role in the association of PSII and LHCII in supercomplexes.

7. Excitation energy transfer and primary charge separation

7.1. Energy transfer within the core or peripheral antenna

The kinetics of excitation energy transfer relies on the energy transfer from pigment to pigment within the core and peripheral core antenna complexes and the energy transfer between various pigment–protein complexes. The excitation energy can disappear from the system by the primary charge separation reaction or by the natural lifetime of the excited state, and can reappear in the system by recombination of the charged pair. Together, these processes determine the dynamics and extent of primary charge separation.

The energy transfer between chlorophylls within the core and peripheral antenna complexes can be described reasonably well by the Förster approach (see, e.g. [125]). This approach relies on small interactions between chlorophylls and may therefore not be valid for the strongly coupled red chlorophylls, but between red and bulk and among all bulk chlorophylls this approach seems justified. It has experimentally been shown by fluorescence upconversion that equilibration of excitation energy among chlorophylls occurs in a few hundred ps, both in the PSI core complex and in LHCI [126,127]. The equilibration among the red chlorophylls within each complex occurs in about 2–6 ps [128,129].

7.2. Energy transfer from the peripheral antenna to the core complex

The energy transfer among chlorophyll–protein complexes is considered to occur in the tens of picosecond time range [130], but quite some variation may occur in several systems. The rate of energy transfer depends on the distance between pigments according to the Förster approach by the power of 6, so the presence of a pigment between two other pigments will have an enormous effect on the rate. In this respect, the discovery of linker chlorophylls between the core and peripheral antenna complexes of plant PSI is very important for the description of the overall kinetics [3], and modeling on the basis of crystal structure of green plant PSI suggested energy transfer of LHCI to PSI in just a few ps [131]. Furthermore, modeling of the kinetics in the $(PSI)_3(IsiA)_{18}$ complex of *Synechocystis* suggested energy transfer from IsiA to PSI in a few ps [132,133]. It is possible that also in this system there are chlorophylls present between the two pigment systems, thus allowing fast energy transfer. For the PSI–LHCII complex of green plants in state 2 no direct data are available. However, an analysis of unstacked and native thylakoids membranes, an additional energy transfer from LHCII to PSI was found to occur within 25 ps [134].

7.3. Trapping of excitation energy by charge separation

When the excitation energy arrives at the primary electron donor P700 (a special pair of Chl *a* and Chl *a*′ molecules [10]), the excited state can be converted into a charge separated state, resulting in oxidized P700 and a reduced primary electron acceptor A_0. The charge separation is stabilized by fast electron transfer from the first acceptor to secondary acceptors. It is possible that, like in the purple bacterial reaction center [135] and in PSII [136], the charge separation starts on an accessory chlorophyll molecule [137]. If the primary electron donor would be the only chlorophyll in the complex, the rate of the intrinsic charge separation reaction can be measured directly. In a system with more chlorophylls, the intrinsic rate has to be multiplied with the probability that the excited state resides on the electron donor. In one limiting case, the energy transfer processes among the antenna chlorophylls are considered much faster than the rate of primary charge separation (trap-limited kinetics). In this case, the rate of charge separation is correlated with the size of the antenna, and is also correlated with the numbers and energies of red chlorophylls in the antenna [138]. A variation is the situation in which the rate to the primary donor is rate-limiting (transfer-to-the-trap limited kinetics). Another possibility is that the energy migration in the antenna determines the overall charge separation rate (diffusion-limited kinetics). Nowadays, most authors consider the charge separation in the PSI core complex as either trap-limited or transfer-to-the-trap limited or both [139,140]. In the PSI–LHCI complex the presence of large numbers of connecting chlorophylls between peripheral and core antenna [3] also would suggest fast energy transfer between peripheral and core antenna and thus either trap-limited or transfer-to-the-trap limited kinetics, but here the situation is more complex (see below). In the PSII-containing grana membranes, the kinetics is thought to be at least in part diffusion-limited [141].

It has been shown that the main phase by which excitations disappear from the PSI core complex by charge separation is between 20 and 50 ps [67,69]. These lifetimes depend on the numbers and energies of red chlorophylls in the system: in complexes with only small amount of red chlorophylls this lifetime is close to 20 ps, in PSI trimers of *Spirulina* with strongly red-shifted red chlorophylls this lifetime is about 50 ps [67]. A doubling of the size of the antenna by the addition of 18 IsiA complexes in a ring around the PSI trimer results also in a

doubling of the main trapping time [132,133], consistent with both trap-limited and transfer-to-the-trap-limited kinetics and fast energy transfer between core and peripheral antenna. Holzwarth and co-workers suggested, based on measurements on PSI core complexes isolated from wild-type and site-directed mutants of *C. reinhardtii*, a more complex charge separation scheme [137,140,142] by assuming a first, fully reversible and very fast charge separation reaction, followed by an electron transfer reaction that stabilizes the charge separation. In their view, the first charge separation reaction occurs in about 6–9 ps, while the about 20 ps kinetics is determined by the secondary electron transfer reaction. A small amount of trapping in 6–9 ps has been confirmed by other groups (see, e.g., [143]), but it is hard to understand how the kinetics of the ~20 ps can arise from a secondary electron transfer step if the kinetics of this phase depend strongly on the numbers and energies of red chlorophylls in the core antenna. Another possibility is that the small 6–9 ps phase arises from excitations that are absorbed by the chlorophylls close to or in the central reaction center part, which can give rise to a relatively fast charge separation because these excitations do not have to pass large numbers of chlorophylls in the core complex.

In PSI–LHCI complexes from green plants, the charge separation kinetics is more complex and consists of at least two trapping phases with different spectral features [66,144,145]. The first main trapping phase occurs in about 20–25 ps, is characterized by a decay-associated spectrum that peaks at about 685 nm and has a shoulder around 720 nm, and resembles the decay-associated spectrum of a PSI core complex with small amounts of red chlorophylls, like the monomeric PSI complex of *Synechocystis* PCC 6803 [69] and the PSI complex of *Synechococcus* PCC 7942 [133]. Experiments on PSI–LHCI complexes obtained from *Arabidopsis* lines with antisense constructs against Lhca proteins revealed equal amplitudes and spectra of this phase [143]. These observations suggest that the 20–25 ps phase arises from excitations that are absorbed and trapped in the PSI core complex. The second main trapping occurs in about 60–120 ps and is characterized by a decay-associated spectrum with peaks of about equal amplitude at about 685 nm and 725 nm [66,144]. The amplitude of this phase decreases proportionally when smaller amounts of Lhca proteins are bound to PSI cores from *Arabidopsis* plants with antisense constructs against various Lhca proteins [143]. This phase is therefore attributed to excitations that were absorbed in the peripheral antenna and slowly transferred to the core complex.

The excitation decay in PSI–LHCI complexes from *C. reinhardtii* is also characterized by two main decay phases [144,146], but here the second phase is faster than the second phase in green plants. The LHCI antenna of *Chlamydomonas* contains more LHCI proteins, but less red chlorophylls, so the amount and energies of red chlorophylls have a larger effect on the excitation trapping than the antenna size.

It is not clear why the excitation decay in PSI–LHCI complexes has a 'slow' phase of around 100 ps. The presence of many linker chlorophylls between peripheral and core antenna should in fact result in fast energy transfer between both complexes. A possible explanation may be given by slow energy transfer processes within at least some of the Lhca proteins (see, e.g., [70,147]) and/or by special properties of the red-most chlorophylls in the Lhca proteins (see Section 5.2.2).

7.4. Trapping of excitation energy by increased nonradiative decay

A study on reconstituted Lhca proteins has revealed that the main decay lifetimes are about 1.4 and 3.4 ns for Lhca1 and Lhca3, and about 0.8 and 3.1 ns for Lhca2 and Lhca4 [148]. These lifetimes seem to depend on the pigment composition (Lhca1 and Lhca3 are enriched in Chl *a*, Lhca2 and Lhca4 in Chl *b*—see Section 5.2.1) are shorter than those of the LHCII and Lhcb4 proteins of PSII, but not short enough to affect the excited state lifetime of the PSI–LHCI complex to a significant extent, because the trapping of excitation energy by charge separation occurs in less than 100 ps (see above). This situation is different in PSI–IsiA complexes from iron-starved cyanobacteria, because the excited state lifetime of small IsiA aggregates has been shown to be about 65 and 210 ps [149]. In this case, the decay of the excited state competes with the decay by charge separation, and will become important in PSI complexes with many bound IsiA subunits [150]. Both in Lhca and in IsiA, the shorter lifetimes are thought to be caused by increased rates of nonradiative decay, resulting in an increased dissipation of excitation energy into heat. It is possible that some energy losses occur near the red chlorophylls in LHCI [147] and thus that the red chlorophylls have a photoprotective role (see also Section 5.2.3). A photoprotective role has also been suggested for the extremely red-absorbing chlorophyll(s) in PSI trimers of *Spirulina platensis* [68].

8. Regulation

Plants are able to adapt photosynthesis to changes in light quality and quantity. The activity of photosynthesis including PSI is regulated at multiple levels. The reversible association of LHCII with PSI, mentioned in Section 6, adjusts in the short term PSI activity to imbalances in the inter-photosystem distribution of excitation energy and is triggered by posttranslational protein modification. In the long-term, imbalances in inter-photosystem energy distribution are counteracted by adjustment of photosystem stoichiometry, which requires a signalling network that coordinates photosynthetic gene expression in plastids and nucleus. For the regulation of the expression of nuclear photosynthetic genes, plastid-to-nucleus (retrograde) signalling is thought to modulate nuclear gene expression in response to altered environmental conditions relevant for plastid functioning.

8.1. Acclimatory responses

Changes in light conditions are thought to result in altered expression of plastid and nuclear genes and ultimately in the adjustment of photosystem stoichiometry to the altered environmental conditions [151–153]. Recent studies have

proposed a functional relationship between the short-term responses – LHCII phosphorylation and state transitions – on the one hand, and the long-term response to altered light conditions on the other [154,155]. Bellafiore et al. [116] demonstrated that the growth of *stn7* mutant plants is affected by rapidly changing light conditions. Under conditions of well-defined changes in the spectral composition of light, Bonardi et al. [117] showed that STN7 is necessary for the long-term adaptation of the photosynthetic apparatus and for the transcriptional regulation of several nuclear and plastid photosynthetic genes. This provides a clear molecular link between the short- and long-term responses to changes in light conditions. One can only speculate on how STN7 triggers changes in gene expression in the nucleus, but three hypotheses were suggested: (i) the phosphorylation state of LHCII directly provides information for plastid-to-nucleus signalling; (ii) an unknown protein is phosphorylated by STN7 and participates in plastid-to-nucleus signalling; and (iii) state transitions and the associated conformational changes in thylakoids stimulate signalling to the nucleus [17].

8.2. Retrograde signalling

Early evidence that nuclear genes are regulated by plastid-derived signals came from studies of photo-oxidized plants, which showed decreased expression of nuclear photosynthetic genes. Regulation occurs frequently at the transcriptional level, and the *Lhcb* genes, coding for light-harvesting proteins of photosystem II, were found to be the most down-regulated. The transcriptional response of selected nuclear photosynthetic genes to variation in the excitation state of the two photosystems showed that the redox state of the plastoquinone pool controls the activity of the plastocyanin promoter [153]. The *Arabidopsis* "chlorophyll *a*/*b* binding protein underexpression" mutant *cue1* offered additional evidence for the involvement of the redox state of the plastoquinone pool in the regulation of nuclear photosynthetic genes [156]: lack of the *CUE1*-encoded phosphoenolpyruvate/phosphate translocator (PPT1) in the inner chloroplast envelope results in a reduced flux through the shikimate pathway and in a decrease in the plastoquinone pool, associated with an altered redox state of the thylakoids. The redox state of the stromal electron acceptors of PSI, in fact, seems to be crucial for the regulation of *Lhcb* genes [155].

In addition to redox signalling, the chlorophyll biosynthetic pathway has been associated with the control of nuclear gene expression. In *Chlamydomonas*, intermediates in the chlorophyll biosynthetic pathway modulate the accumulation of transcripts of several nuclear chloroplast genes [157,158]. *Arabidopsis* mutants that do not react to norflurazon-induced photo-oxidative damage by repression of *Lhcb* transcription (*genomes uncoupled 1–5*: *gun1–5*) were affected in genes encoding proteins involved in tetrapyrrole metabolism: the products of *GUN2*/*HY1* and *GUN3*/*HY2* contribute to heme degradation in the "Fe branch" of tetrapyrrole biosynthesis [158], *GUN5* encodes the CHL H subunit of the Mg-chelatase [159], and GUN4 binds product and substrate of Mg-chelatase, and activates Mg-chelatase [160].

A third retrograde signalling pathway depends on the expression of plastid genes [161]; nuclear photosynthetic genes are down-regulated when plastid translation is impaired and genetic analyses indicate that also the mitochondrial translation rate has an effect on the expression of nuclear photosynthetic genes [162].

Components of the three plastid-to-nucleus signalling pathways, their interdependence, as well as the entire sets of their nuclear target genes remain largely unknown and require further genetic and biochemical experiments. Studies of the nuclear chloroplast transcriptome imply a further level of complexity; at least two distinct types of transcriptional regulation exist in flowering plants: a master switch, acting in a binary mode by either inducing or repressing the same large set of genes [163] and a second mechanism responsible for the specific co-regulation of nuclear genes for photosynthesis and for plastid gene expression [164]. The discovery that STN7 is required for photosynthetic acclimation [117] provides a first protein component in the link between the thylakoid redox state, retrograde signalling and changes in nuclear photosynthetic gene expression.

8.3. PSI proteins in a whole-plant perspective

Are all PSI subunits necessary for the plant? Here evolutionary arguments are not fully consistent with genetic data. If gene products would not have an important function, the probability that the corresponding genes should have undergone mutations eliminating their expression is close to unity. However, the majority of the PSI–LHCI subunits are not essential for growth and photosynthesis of the plant; many can even be removed by genetic manipulations without giving an obvious phenotype. The only possible way to understand such an apparent contradiction is that the experimental conditions that we grow our plants under are different from those where evolution has acted, i.e. in the nature. When a strategy to measure the contribution to the Darwinian fitness of individual gene products under field conditions was developed [165], this could also be applied to PSI proteins. Although relatively few have so far been tested, the general pattern seem to be that under field conditions, even proteins that can be removed without causing a measurable phenotype in the laboratory seem to be useful for the plant under natural conditions [166]. The importance of state transitions (see Section 6) have also been measured in a similar way, and it seems as if the *stn7* mutant, lacking state transitions, have a reduced fitness although not to the same extent as plants lacking the qE type of NPQ [167].

Novel tools that have been developed in genomics hold big promise to help elucidating the molecular functions of individual proteins or cofactors, since genes induced or repressed, or other proteins or metabolites that accumulate or disappear, when a given gene is deleted could of course be informative in understanding the molecular lesions resulting from the manipulation. Although these techniques now have started to be used on photosynthetic proteins, this research field is still in the cradle.

9. Conclusions and perspectives

A large body of knowledge about the structure and function of photosystem I have been gained during the last 4–5 years: the structure of PSI–LHCI at 4.4 Å, the discovery of new subunits, the structure of a PSI–LHCII complex, molecular basis for the red chlorophylls, transfer of excitation energy within the PSI–LHCI complex and the regulation of nuclear genes with function in the thylakoid membrane. However, numerous unanswered questions remain and new questions have emerged.

The additional densities in the PSI–LHCII supercomplex should be established. Blue-native polyacryl gel electrophoresis (BN-PAGE) seems to be a possibility to separate the labile PSI–LHCII complexes from other supercomplexes [29] and additional mass spectrometry or immunoblotting could establish the presence or absence of the PsaO and PsaP subunits and other possible components of the PSI supercomplexes.

For structural studies on multi-subunit complexes the use of a thermophilic organism has often been crucial, which is exemplified by the cyanobacterial PSI structure from *Thermosynechococcus elongatus* [10]. The model plant *A. thaliana* with C3 anatomy and metabolism has an optimal growth temperature of about 20 °C. On the other hand, plants with C4 metabolism have enhanced tolerance to high temperature compared to C3 plants. Maize leaf photosynthesis is not inhibited until leaf temperature approaches about 40 °C [168]. Thus some of the future structural work should be performed on plants like maize, which are supposed to have more stable photosystem I and II supercomplexes.

In many C3 plants PSI is prone to photodamage under certain conditions: the combination of chilling temperatures and moderate light intensities result in preferential damage to PSI (reviewed in [169]). This inhibition requires oxygen and electron transfer [170] and involves oxidative destruction of the FeS clusters in PSI. PSI subunits are damaged during inhibition; however the repair seems different from what is known from repair of the D1 subunit during PSII photoinhibition. Apparently the entire damaged PSI core complex is degraded although not much is known about the steps in the turnover process or which proteases are involved. Thus, future research should be directed towards identifying the proteases involved and enzymes involved in breakdown or reuse of chlorophylls and possible other co-factors. Similarly, assembly of new PSI complexes with all its co-factors are also poorly understood and deserves much more attention in the future.

PSI damage is likely to be a very important factor for limiting crop growth in temperate climates. A greater understanding of PSI function, inhibition, turnover and assembly will thus form the basis for improving yield for the benefit of mankind both as a food and feed source but equally important for the future exploitation of photosynthesis for energy purposes.

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